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## Conversion of Somatic Antigens in *Salmonella* by Phage Infection Leading to Lysis or Lysogeny<sup>1</sup>

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The conversion of *Salmonella anatum* (group E<sub>1</sub>, somatic antigens 3, 10) to group E<sub>2</sub> (antigens 3, 15) by infection with phage  $\epsilon^{15}$  and its virulent mutant  $\epsilon^{15}$  vir has been analyzed. Antigen 15 appears in the cells within a few minutes after infection with either phage. The receptors for another phage,  $\epsilon^{34}$ , which can be adsorbed only by cells with antigen 15, appear with the new antigen. The receptors for phage C<sub>441</sub>, which is adsorbed only by cells with antigen 10, disappear following infection. In the early stages the cells have both antigens 10 and 15 and both sets of phage receptors. The cells that survive infection segregate lysogenic and nonlysogenic cells in their progeny. In the nonlysogenic segregants the changes in antigenic structure and in phage receptors initiated by phage infection persist for several generations; later, the cells revert to the typical E<sub>1</sub> condition. The results indicate that the converted characters are controlled by vegetative phage as well as by prophage. Their bearing on the mechanism of conversion is discussed.

### INTRODUCTION

Organisms of the genus *Salmonella* undergo changes in somatic antigens upon lysogenization with certain phages (Iseki and Sakai, 1953b; Uetake *et al.*, 1955; Terada *et al.*, 1956; Uetake, 1956, 1957; Harada, 1956; Nakagawa, 1957). Among these changes is the conversion of strains belonging to the group E<sub>1</sub>, with O antigens 3, 10, to group E<sub>2</sub>, with O antigens 3, 15 when they become lysogenic for phages carried by organisms of groups E<sub>2</sub> and E<sub>3</sub>. Reversion from E<sub>2</sub> to E<sub>1</sub>, with loss of lysogeny, is observed by cultivation of E<sub>2</sub> cells in media containing anti-15 serum. In fact, most of the naturally occurring E<sub>2</sub> organisms are the exact lysogenic counter-

parts of known E<sub>1</sub> organisms; and the inference seems justified that the same conversions observed under laboratory conditions also occur in the natural habitat (Uetake *et al.*, 1955).

The appearance of new cell properties, such as antigens, in lysogenized strains has been named "lysogenic conversion" (Lederberg, 1955). It is generally assumed that it expresses the function of the prophage integrated in the genetic material of the host. We describe in this paper a system which permits us to study the early manifestation of new properties following phage infection and the correlation between the presence of such properties and of phage elements in the bacterial cell.

Specifically, using a phage that converts group E<sub>1</sub> organisms to group E<sub>2</sub>, we have studied not only the antigenic factors 10 and 15, which are shifted in the conversion, but also the sensitivity of the infected cells to two unrelated phages, which are adsorbed only by cells with antigen 10 or antigen 15 respectively. The results show that the phage-induced changes appear within minutes after infection, can persist phenotypically in cells that have segregated away the genetic determinants of phage production, and are produced equally well in cells that give a productive, lytic response to infection as in cells that give a reductive, lysogenic response. Thus, the changes in surface properties of the cells are the expression of the phage material acting as a genetic determinant in all its forms.

### MATERIALS AND METHODS

*The experimental system.* The basic experimental system to be described consists of *Salmonella anatum* 293 (= strain A) and of phage  $\epsilon^{15}$  active upon it. Upon infection with  $\epsilon^{15}$ , the surviving cells of A give rise to lysogenic progeny A( $\epsilon^{15}$ ). Strains A( $\epsilon^{15}$ ) differ from A in somatic antigens. Strain A is a typical E<sub>1</sub> group *Salmonella*, with somatic antigens 3, 10. Strain A( $\epsilon^{15}$ ) is a typical E<sub>2</sub> group *Salmonella*, with somatic antigens 3, 15.

In addition, strain A( $\epsilon^{15}$ ) is unable to adsorb certain phages, including  $\epsilon^{15}$  itself and an unrelated phage C<sub>341</sub>, which can infect strain A; instead, strain A( $\epsilon^{15}$ ) is sensitive to another unrelated phage  $\epsilon^{34}$ , which is not adsorbed by A. These properties are listed in Table 1. The experiments to be described investigate the appearance and persistence of the above differential characters in cells of strain A infected with phage  $\epsilon^{15}$  and in their progeny.

*Bacterial strains.* All *Salmonella* strains used are international standard

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TABLE 1  
PROPERTIES OF *Salmonella anatum* STRAIN A AND ITS LYSOGENIC DERIVATIVES

Strain	O-Antigens	Adsorption of phages		
		$\epsilon^{15}$ or $\epsilon^{15} \text{ vir}$	GMI or CMI	$\epsilon^{34}$ or $\epsilon^{34} \text{ vir}$
A	3, 10	+	+	—
A ( $\epsilon^{15}$ )	3, 15	—	—	+
A ( $\epsilon^{15}$ , $\epsilon^{34}$ )	3, 15, 34	—	—	—

strains obtained from the Enterobacteriaceae Committee of Japan. Strain A is *S. anatum* 293 (group E<sub>1</sub>, antigens 3,10: e,h-1,6 . . .). It gives an appreciable proportion of rough variants, which fail to adsorb phage and may complicate the serological tests. For each experiment, a single smooth colony was picked into broth and grown to the desired concentration. Under these conditions only about 0.1 to 0.2% rough cells were present, a proportion that does not interfere with the experiments. The experimental cultures were adjusted to the desired titer by colorimetric test and assayed by colony count. The generation time of strains A and A( $\epsilon^{15}$ ) growing in a water bath at 37° is 30 minutes. A streptomycin-resistant derivative AS<sup>r</sup> was obtained for use in the assay of free phage (Bertani, 1951).

**Phage strains.** Phage  $\epsilon^{15}$  is a temperate phage isolated from *S. newington* C<sub>2</sub> (group E<sub>2</sub>, antigenic structure 3,15: e,h-1,6 . . .) and propagated on *S. anatum* A (Uetake *et al.*, 1955). The latent period of this phage on A is 55 minutes at 37°; the burst size is about 400. At a multiplicity of infection of 5, 20 to 40% of the infected cells survive and give rise to lysogenic progeny A( $\epsilon^{15}$ ), with the antigenic structure: 3,15: e,h-1,6 . . . The frequency of liberation of phage  $\epsilon^{15}$  from A( $\epsilon^{15}$ ) is of the order of 10<sup>-4</sup> per cell per generation under the standard conditions of these experiments. Strain A( $\epsilon^{15}$ ) is induced to lysis and phage liberation by ultraviolet light.

Phage  $\epsilon^{15} \text{ vir}$  is a virulent mutant of  $\epsilon^{15}$ , which lyses all susceptible cells it infects. Latent period, burst size, and serological properties are the same for  $\epsilon^{15}$  and  $\epsilon^{15} \text{ vir}$ .

Phage C<sub>341</sub> is a virulent mutant of phage g<sub>341</sub>, isolated from *S. thomasville* 341 (Uchida *et al.*, 1956; Uetake, 1957). These phages are adsorbed by most *Salmonella* strains of group E<sub>1</sub>, but not by strains of group E<sub>2</sub>. The adsorption rate constant on strain A is 6 × 10<sup>-9</sup> min<sup>-1</sup> per cell.

Over 150 phage particles per cell can be adsorbed by growing cells of strain A. The latent period is 30 minutes; burst size about 100. Phage C<sub>341</sub> is serologically unrelated to  $\epsilon^{15}$  by cross-neutralization tests.

Phage  $\epsilon^{34} \text{ vir}$  is a virulent mutant of temperate phage  $\epsilon^{34} c$ , which was isolated from *S. thomasville* 341. These phages can infect cells with antigens 3,15; the temperate phage, upon lysogenization, converts the somatic antigens to 3,15,34 (Uetake, 1956; Harada, 1956; Nakagawa, 1957). The adsorption rate constant is 3 × 10<sup>-9</sup> min<sup>-1</sup> per cell. The maximum adsorption capacity is about 25 phages per cell. The latent period is 35 minutes; burst size about 100. Phages  $\epsilon^{34} c$  and  $\epsilon^{34} \text{ vir}$  do not adsorb on strain A (nor on any other strains without antigen 15). They are serologically unrelated to phages  $\epsilon^{15}$  and C<sub>341</sub>. Phage  $\epsilon^{34} \text{ vir}$  for our work was propagated on *S. anatum* A *do*, a defective derivative of strain A( $\epsilon^{15}$ ) that has antigen 15 but liberates no phage  $\epsilon^{15}$  (Uetake, to be published). This avoids the presence of any  $\epsilon^{15}$  phage in lysates of  $\epsilon^{34} \text{ vir}$ . Phage stocks were prepared in aerated nutrient broth cultures or by the agar layer method.

**Media.** Difco nutrient broth (8 g/liter in demineralized water, plus 5 g/liter NaCl) was used throughout. Platings were made on nutrient agar (1.1% and 0.65% agar in nutrient broth for bottom and top layers, respectively) except for viable cell counts, which employed a tryptone-yeast extract-glucose agar.

**Serological tests.** Antibacterial antisera were prepared by standard methods (Kauffmann, 1954). Anti-10 serum was obtained by absorbing O-antisera against strain A with cells of A( $\epsilon^{15}$ ); anti-15 serum by absorbing antiserum against A( $\epsilon^{15}$ ) with cells of A. Test for antigens in colonies was done by slide agglutination, using anti-10 and anti-15 sera.

Tube agglutination tests were done using 0.2 ml of serum dilution in saline plus one drop of cell suspension in 7-mm tubes; readings were done after incubation for 2 hours in a water bath at 50° and overnight at room temperature. Cells from experimental cultures were heated for 4 minutes in boiling water, centrifuged and resuspended in saline for use as antigens.

Antiphage sera were prepared in rabbits by subcutaneous inoculations followed by intravenous injections. The sera used had the following titers: anti- $\epsilon^{15}$ —80 min<sup>-1</sup>; anti-C<sub>341</sub>—750 min<sup>-1</sup>; anti- $\epsilon^{34} \text{ vir}$ —70 min<sup>-1</sup>. The sera were used without previous absorption of antibacterial antibodies. In the experiments, possible agglutinating action of antibacterial

antibodies in antiphage sera was prevented by using low bacterial concentrations, and interference by these antibodies with phage adsorption was tested in control experiments. There is no cross reaction between the antigens of strain A and of the phage  $\epsilon^{15}$  detectable by phage neutralization tests.

*Tests for phage-carrying colonies* were done by replica plating (Lederberg and Lederberg, 1952) using velveteen pads. The colonies to be tested were replicated onto plates with a 4-ml overlay of 1.1% nutrient agar containing indicator cells. The indicator plates (which could be stored in the refrigerator) were dried open for 30 minutes at 37° before replication.

*Tests for killing of cells by phages C<sub>341</sub> and  $\epsilon^{34}$  vir.* The cells to be tested were exposed at a density of about  $10^5$  cells per milliliter to phage with a titer of  $10^8$  particles per milliliter. During exposure to phage the mixtures were kept at 20° to reduce bacterial multiplication. At intervals, samples were diluted 1:5 in a mixture of anti-C<sub>341</sub> serum (1:100) and anti- $\epsilon^{34}$  serum (1:50), kept at 37° for 5 minutes, and plated for viable counts, adding to each plate one drop of the same mixture of sera, to minimize reinfection of surviving cells on the plates. A uniform serum treatment was used in tests for killing by phage C<sub>341</sub> alone, by  $\epsilon^{34}$  vir alone, or by a mixture of the two. Suitable controls indicated that the killing rates were not affected by the presence of anti- $\epsilon^{15}$  serum, which was also present in the experimental mixtures.

*Tests for killing of cells by phage  $\epsilon^{15}$  vir* were done in a similar manner, except that serum anti- $\epsilon^{15}$  was either omitted from the experimental mixtures or, if present, was rendered ineffective by suitable dilution before testing. Anti- $\epsilon^{15}$  serum was used on the plates.

*Tests for adsorption of phages* by cells of strain A infected with  $\epsilon^{15}$  were done by chilling, centrifuging, and resuspending the cells to the desired density. The details are described in the appropriate section.

## RESULTS

*The basic experiment.* In a typical experiment, a culture of strain A from a single smooth colony was grown with aeration to a density of about  $2 \times 10^8$  cells per milliliter. Equal volumes of the culture and of phage  $\epsilon^{15}$  at a titer of  $1 \times 10^9$  particles per milliliter were mixed, and the mixture was kept at 37° without aeration. After 10 minutes, when over 95% of the phage was adsorbed, the mixture received an equal volume of anti- $\epsilon^{15}$  serum diluted 1:50. After another 10 minutes, a 1:50 dilution

was made in broth containing anti- $\epsilon^{15}$  serum diluted 1:400.<sup>3</sup> At 90 minutes, a further dilution 1:8 was made in the same medium, and a further dilution 1:120 was made at 180 minutes. Thus the cells were kept at low density and reinfection with phage  $\epsilon^{15}$  was prevented by the antiserum. Parallel dilutions without serum were used for determination of unadsorbed phage, latent period, and burst size. Samples were taken at intervals and tested in the following ways:

1. Viable cells, by colony count.
2. Cells carrying phage  $\epsilon^{15}$ , by replication of colonies onto indicator plates.
3. Antigens 10 or 15 in colonies, by slide agglutination.
4. Presence of antigens 10 and 15, by tube agglutination after heat-killing the cells and resuspending them in saline.
5. Killing of the cells by phage C<sub>341</sub>.
6. Killing of the cells by phage  $\epsilon^{34}$  vir.
7. Killing of the cells by a mixture of phages C<sub>341</sub> and  $\epsilon^{34}$  vir.
8. Killing of the cells by phage  $\epsilon^{15}$  vir.
9. Ability of the cells to adsorb phages C<sub>341</sub> and  $\epsilon^{34}$  vir, and rates of phage adsorption.<sup>4</sup>

Not all these properties were tested in each experiment at all time intervals; but enough duplications were made and the results were reproducible enough to permit us to draw a composite picture. A typical experiment is detailed in Fig. 1.

The most variable feature is the proportion of infected cells that survive infection, which varies between 20 and 40% in different experiments as measured at 20 minutes after infection. This variability only gives a factor 2 of uncertainty in bacterial titers, which does not interfere with the planning of dilutions. The infected survivors resume multiplication within 30 minutes after infection and multiply at the same rate as uninfected bacteria.

We shall discuss individually the evolution of the various properties of these surviving bacteria (see Table 2) and then give a general picture

<sup>3</sup> The anti- $\epsilon^{15}$  serum used in these experiments could not be absorbed with A or A( $\epsilon^{15}$ ) cells to remove antibacterial antibodies because such absorption left in the serum some materials that interfered with adsorption of other phages by the cells. Clumping of the cells was prevented by keeping them at low concentration and by careful resuspension before using them.

<sup>4</sup> This effect of antiserum on the adsorption rate of  $\epsilon^{34}$  vir is probably due to the presence of antibacterial antibodies in the antiphage serum.

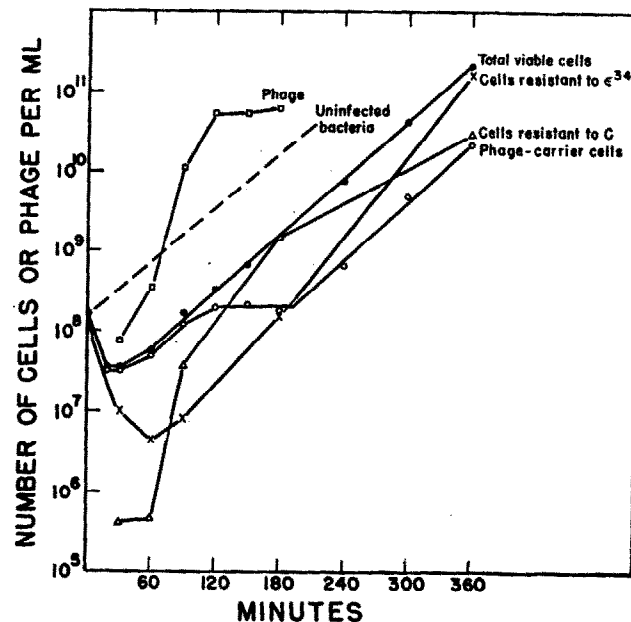


FIG. 1. A typical experiment of infection of strain A with phage  $\epsilon^{15}$ . The curve of the colony counts of uninfected cells was obtained from a portion of the culture that received no phage. The phage plaque counts were measured on a sample diluted in broth without anti- $\epsilon^{15}$  serum after the first 10 minutes of serum treatment. The phage-carrier cells were determined by replica plating. The values for cells resistant to phages  $\epsilon^{34}$  *vir* and to  $C_{341}$ , respectively, correspond to measurements after 20 or 30 minutes of exposure to the test phages. Abscissa: time after infection. Ordinate: values referred to the initial phage-cell mixture.

of this evolution and an interpretation of the genetic functions of phage  $\epsilon^{15}$ .

*Carrier state, lysogeny, and segregation of nonlysogenic progeny.* When the colonies formed by the surviving bacteria are tested by replication on an  $\epsilon^{15}$ -sensitive indicator, at first practically all colonies are phage carriers and, if picked and restreaked, contain lysogenic bacteria. As the bacteria multiply, the number of phage-carrier colonies does not increase as rapidly; indeed, after an initial rise the number remains approximately constant for 2 or 3 generations, so that their proportion decreases from nearly 100% to about 10%. Thereafter, the number of phage-carrier colonies begins to increase and rises in parallel with the total cell count (see Table 2 and Fig. 1). This indicates that, following in-

TABLE 2  
PROPERTIES OF THE PROGENY CELLS FROM BACTERIA INFECTED WITH PHAGE  $\epsilon^{15}$

Time after infection (min)	Phage-carrier colonies (%)	Cells killed by phage $C_{341}$ (%)	Cells killed by phage $\epsilon^{34}$ <i>vir</i> (%)	Cells killed by a mixture of $C_{341}$ and $\epsilon^{34}$ <i>vir</i> (%)	Cells killed by phage $\epsilon^{15}$ <i>vir</i> <sup>a</sup> (%)
30	98	99 <sup>b</sup>	74 <sup>b</sup>	96 <sup>b</sup>	
60	85	99 <sup>b</sup>	92 <sup>b</sup>	71 <sup>b</sup>	
90	75	78	95	97	0
120	61	59			
150	30				
180	12	0	90	90	46
300	12				
360	11	85	7	96	

<sup>a</sup> Tested on separate samples without anti- $\epsilon^{15}$  serum.

<sup>b</sup> These values are for an exposure of 20 minutes to the test phage at 20°. All other values are for a 30-minute exposure.

NOTE: All values in the table are from a single experiment, except the one in italics.

fection, the surviving cells segregate out for several generations a mixture of sensitive cells and phage-carrier cells. Such segregation has been observed with *Escherichia coli* infected with phage  $\lambda$  (Lieb, 1953) and with *S. typhi murium* infected with phage P22 (Levine, 1957).

The pattern of segregation was confirmed by single clone experiments, in which at 10 minutes an infection mixture, prepared as usual, was diluted in broth to a concentration of about 2 surviving cells per milliliter and 0.2-ml samples were distributed into individual tubes. After incubation for about 3 hours, the contents of each tube were tested for free phage  $\epsilon^{15}$  by spot test on streptomycin agar seeded with strain AS<sup>r</sup>; then, after addition of anti- $\epsilon^{15}$  serum the samples were plated for colonies on separate plates. All plates with colonies were replicated for phage-carrier test. The results are shown in Table 3.

These experiments show that the presence of lytic responses (free phage) and of surviving cell clones arise by independent events, since their joint frequency is similar to the product of the frequencies of the two individual events (50% plates with phage; 39% plates with cells; 19% plates with both). There is an excess of plates with nonlysogenic cells only over the number expected from the fact that in the experiments of Table 3 early platings contain almost only phage-carrier colonies. This suggests that under the conditions of the single clone experi-

TABLE 3  
SEGREGATION OF NONLYSOGENIC CELLS IN THE PROGENY OF CELLS INFECTED  
WITH PHAGE  $\epsilon^{15}$

Free phage	Plates with Colonies		Number of plates	Number of colonies		Average number of colonies per plate		
	Phage- carrier	Non- carrier		Carrier	Noncarrier	Carrier	Non- carrier	Total
-	-	-	32					
-	+	-	0					
-	-	+	8		245		30.6	30.6
-	+	+	10	78	765	7.8	76.5	84.3
+	-	-	29					
+	+	-	0					
+	-	+	11		282		25.5	25.5
+	+	+	10	80	549	8.0	54.9	62.9
			100	158	1841			
Total: 1999								

Plates with free phage (bursts) = 50

Plates with colonies = 39

Plates with phage-carrier colonies only = 0

Plates with noncarrier colonies only = 19

Plates with both types of colonies = 20

Average number of bacterial cells per tube (lysed or survivors) = 1.14

Average number of bacterial clones per plate = 0.49

Average number of clones with carrier cells per plate = 0.22

Average number of clones without carrier cells per plate = 0.27

Approximate proportion of  
phage-carrier colonies in  
plates with colonies of both  
types

	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	
Number of plates	2	3	7	5	2	1	Total: 20

NOTE: A mixture of A cells ( $1.2 \times 10^8$ /ml) and phage  $\epsilon^{15}$  ( $5.0 \times 10^8$ /ml) was kept at  $37^\circ$  for 10 minutes, diluted  $1:2 \times 10^7$  in broth, and distributed in 0.2-ml samples in 100 tubes. The tubes were incubated for 170 minutes at  $37^\circ$ , then placed in a water bath at  $10^\circ$ . Each sample was tested for free phage  $\epsilon^{15}$  by spot test (about 0.01 ml) on a streptomycin-agar plate seeded with cells AS<sup>r</sup>. One drop of a 1:50 dilution of anti- $\epsilon^{15}$  serum was added to each tube, and the tubes were returned to  $37^\circ$  for 10 minutes, then again to  $10^\circ$ . The contents of each tube were plated by spreading on individual agar plates. All plates with colonies were counted and replicated for phage-carrier test.

ment some infected cells give rise to few lysogenic progeny cells or none at all. This is supported by findings in some preliminary experiments with single cell isolation pedigrees (carried out Dr. D. K. Fraser), in which some clones at the early cell divisions gave repeated cell deaths without phage liberation. The possibility of a frequent early death of potentially lysogenic progeny cells is also suggested by the significantly lower colony counts in those plates that have only nonlysogenic colonies, compared with the counts in plates with lysogenic colonies (see Table 3).

The most relevant finding shown in Table 3 is that all clones containing lysogenic colonies also contain nonlysogenic, sensitive cells. This confirms the existence of segregation. The average number of cells per clone is 37 (about 5.2 generations). The proportion of lysogenic colonies in plates with lysogenics was  $158/1313 = 13\%$ ; the proportion of lysogenics among all colonies was  $158/1841 = 8.5\%$ . These values are in agreement with the final proportion of about 10% lysogenics found in the mass growth experiments. The frequency distribution of the proportion of lysogenics in the plates, ranging from  $\frac{1}{2}$  to  $\frac{1}{64}$ , is an index of the variability of the pattern of segregation, as observed also with phage P22 of *S. typhi murium* (Levine, 1957).

As expected, all phage-carrier colonies gave a positive slide agglutination reaction with anti-15 serum, since they contained lysogenic cells A( $\epsilon^{15}$ ). Conversely, all colonies without phage gave a positive reaction with anti-10 serum and a negative one with anti-15 serum.

*Evolution of antigens 10 and 15 in the infected cells.* Table 4 shows that the infected cells of strain A were already agglutinable by anti-15 serum 15 minutes after infection; they were agglutinated at the full serum titer by 30 to 45 minutes. Additional tests showed some detectable agglutination after 3 to 5 minutes and definite agglutination at 8 minutes. Meanwhile, the agglutinability by serum anti-10 persisted, apparently unchanged. Judging from the completeness of agglutination by either serum, it seems clear that antigens 10 and 15 coexist on the same cells. Note that a mixture of 10% A( $\epsilon^{15}$ ) cells and 90% A cells did not produce visible agglutination with anti-15 serum. Hence, over 10% of the cells must have developed some antigen 15 within 3 to 5 minutes.

A remarkable finding is that the same result is obtained when phage  $\epsilon^{15}$  is replaced by  $\epsilon^{15}$  *vir*, a virulent mutant, which does not lysogenize and which causes 99% of the infected cells to lyse between 60 and 120 minutes after infection (see Table 4). Here too, antigen 15 is detected within less than 10 minutes. This finding indicates that the formation of

antigen 15 can be controlled by the phage in its vegetative state and does not require the presence of an established prophage. Note also that the development of antigen 15 in infected cells is scarcely affected by the multiplicity of infection (between 1.8 and 14).

Table 4 also shows tests with anti-10 and anti-15 sera on cells taken 180 and 360 minutes after infection with  $\epsilon^{15}$ , at which times the proportion of phage-carrying cells has decreased to about 10% (stable lysogenics). The cell suspensions are agglutinated by both sera, but the degree of agglutination by anti-15 serum is stronger at 180 minutes, while agglutination by anti-10 serum is stronger at 320 minutes. These data are not quantitative enough to decide whether the extent of the antigenic reaction with anti-15 corresponds to the proportion of cells that carry phage  $\epsilon^{15}$ ; but, qualitatively, the evolution of antigens parallels the evolution of lysogeny and segregation.

*Appearance and development of susceptibility to phage  $\epsilon^{34}$  vir.* Since phage  $\epsilon^{34}$  vir is not adsorbed by cells of strain A but is adsorbed by A( $\epsilon^{15}$ ),

TABLE 4  
AGGLUTINATION REACTIONS OF CELLS OF STRAIN A INFECTED WITH PHAGE  $\epsilon^{15}$

Antigen	Time after infection (min)	Serum anti-10					Serum anti-15			
		1:200	1:400	1:800	1:1600	1:3200	1:100	1:200	1:400	1:800
Cells A infected with $\epsilon^{15}$	15	+++	+++	+++	++	++	+	+	±	-
	20	+++	+++	+++	+++	++	+	+	+	±
	25	+++	+++	+++	+++	++	++	++	+	+
	30	+++	+++	+++	++	++	+++	+++	++	+
	45	+++	+++	++	++	+	+++	+++	++	+
	60	+++	+++	++	++	+	+++	+++	++	+
	180	++	+	+	+	+	+++	+++	++	+
	360	+++	+++	+++	++	+	++	++	++	±
Cells A infected with $\epsilon^{15}$ vir	15	+++	+++	+++	++	++	+	+	±	-
	20	+++	+++	+++	+++	++	++	++	+	+
	25	+++	+++	+++	+++	++	+++	+++	++	+
	30	+++	+++	+++	++	++	+++	+++	++	+
	45	+++	+++	+++	++	++	+++	+++	++	+
	60	+++	+++	+++	++	++	+++	++	+	+
Cells A uninfected		+++	+++	+++	++	++	-	-	-	-
Cells A( $\epsilon^{15}$ )		-	-	-	-	-	+++	+++	+++	++

killing every infected cell, the receptors for this phage must appear during lysogenization. Their formation can be followed by measuring the killing by  $\epsilon^{34}$  vir of cells that survive infection by  $\epsilon^{15}$ . The results are shown in Table 2 and Fig. 2. The development of susceptibility to  $\epsilon^{34}$  vir is very rapid: 74% of the cells are susceptible after 30 minutes and 90% after 60 minutes. This is consistent with the rapid appearance of antigen 15.

When the rate of killing of cells by  $\epsilon^{34}$  vir is studied, as shown in Fig. 2, it becomes clear that not only the proportion of cells susceptible to phage  $\epsilon^{34}$  vir increases with time up to a maximum, but also the rate at which the cells are killed increases from 0 to a maximum. This rate never quite reaches, for the whole population, the rate of killing for the established lysogenic strain A( $\epsilon^{15}$ ). This is as expected if the receptors were formed slowly and if the rate of killing paralleled the number of effective receptors per cell.

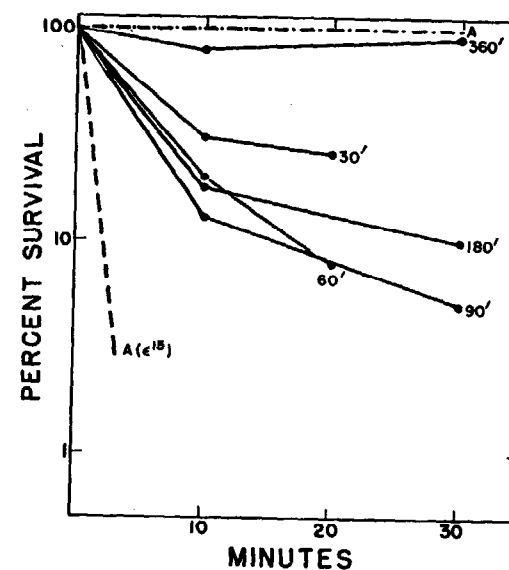


FIG. 2. The survival of bacteria exposed to phage  $\epsilon^{34}$  vir at various times after infection with phage  $\epsilon^{15}$ . Mixtures contained  $10^8$  phage particles and about  $10^8$  cells. The figure near each curve indicates the time between infection with  $\epsilon^{15}$  and challenge with  $\epsilon^{34}$  vir. On the abscissa is the duration of exposure to phage  $\epsilon^{34}$  vir. Curve A: uninfected cells of strain A. Curve A( $\epsilon^{15}$ ): established lysogenic cells.

As time proceeds, the proportion of cells susceptible to phage  $\epsilon^{34}$  *vir* rises first to a maximum of about 95%, then diminishes, until after 360 minutes only about 10% of the cells are sensitive to  $\epsilon^{34}$  *vir*. It is interesting to compare the evolution of the sensitivity to  $\epsilon^{34}$  with that of the phage-carrier state (see above). The comparison can be made by inspection of Table 2. It is clear that the proportion of cells sensitive to  $\epsilon^{34}$  *vir* continues to increase while the proportion of phage-carrier cells is decreasing. The former is still 90% at 180 minutes, when the surviving cells have already undergone 5-6 division cycles and the phage-carrier cells have been reduced to the level of the stable lysogenics for  $\epsilon^{15}$  (about 10%). That the acquisition of sensitivity to  $\epsilon^{34}$  *vir* in noncarrier cells is not permanent is shown by the fact that at 360 minutes, after 6 additional generations, the proportions of  $\epsilon^{15}$  lysogenics and  $\epsilon^{34}$  sensitives are the same. Hence, we conclude that susceptibility to  $\epsilon^{34}$  *vir*, arising under the control of phage  $\epsilon^{15}$ , can persist for several cell generations after the  $\epsilon^{15}$  phage-elements have been removed by segregation. The meaning of this persistence will be discussed in a later section.

*Evolution of susceptibility to phage C<sub>341</sub>.* Since phage C<sub>341</sub> adsorbs only to cells with antigen 10, such as strain A, and not to cells of strain A( $\epsilon^{15}$ ), it is expected that the receptors for C<sub>341</sub>, and hence the sensitivity of the cells to this phage, will be affected during lysogenization by phage  $\epsilon^{15}$ . The findings are shown in Table 2 and Fig. 3.

At 30 and 60 minutes after infection, 99% of the cells were killed by exposure to phage C<sub>341</sub> for 20 minutes, although more slowly at the later time. By 90 minutes, however, only 78% of the cells were killed, and at 180 minutes practically all cells were resistant to phage C<sub>341</sub>. By this time the cells with phage  $\epsilon^{15}$  are only about 10%. At 360 minutes 80% of the cells are again killed by C<sub>341</sub>, but the rate of killing is still definitely lower than for uninfected cells of strain A.

These results indicate that susceptibility to phage C<sub>341</sub> is greatly reduced following infection with phage  $\epsilon^{15}$ , until after about 5-6 generations most of the progeny of the  $\epsilon^{15}$ -infected cells is fully resistant to phage C<sub>341</sub>. The C<sub>341</sub> receptors later reappear in the nonlysogenic progeny; their amounts, however, are not yet normal after 11-12 generations. It is important to note that reappearance of sensitivity to phage C<sub>341</sub> lags far behind the segregation of  $\epsilon^{15}$ -free cells, in the same way as the disappearance of sensitivity to  $\epsilon^{34}$  *vir* does.

*Adsorption of phages C<sub>341</sub> and  $\epsilon^{34}$  *vir*, and evolution of the cell receptors.* Three interpretations of the above findings are possible: either, follow-

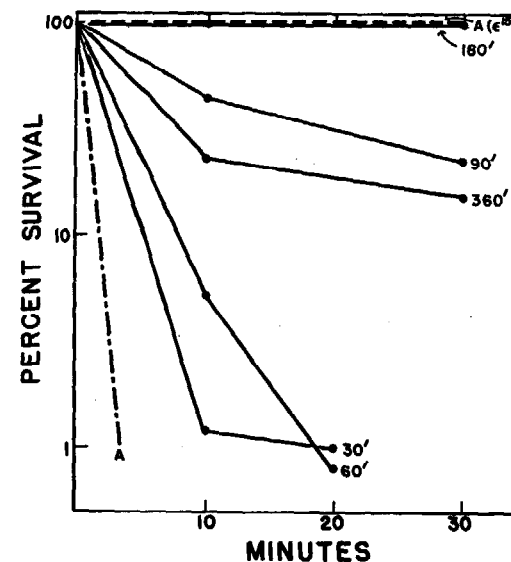


FIG. 3. The survival of bacteria exposed to phage C<sub>341</sub> at various times after infection with phage  $\epsilon^{15}$ . Notations as in Fig. 2.

ing infection with phage  $\epsilon^{15}$ , the receptors for C<sub>341</sub> present in uninfected A cells are blocked, destroyed, or inactivated; or they are preserved but no further synthesized, being diluted among the progeny cells; or they are synthesized at a rate insufficient to keep pace with cell division. The same hypotheses apply to the loss of sensitivity to phage  $\epsilon^{34}$  *vir* in the later evolution of the progeny cells. We have attempted to decide among these possibilities by quantitative tests of adsorption of phages C<sub>341</sub> and  $\epsilon^{34}$  *vir* on progeny cells at various times after infection with phage  $\epsilon^{15}$ .

For these experiments, cells infected as usual with  $\epsilon^{15}$  were diluted in broth with anti- $\epsilon^{15}$  serum to such an extent that at the desired times the bacterial titer would be approximately  $1 \times 10^7$ /ml. At these times, 20-ml samples were taken, assayed for viable count, chilled and centrifuged. The cells were collected in 1.2 ml of cold broth, carefully resuspended and again assayed. These cells, with titers of  $1-4 \times 10^8$ /ml, were used for adsorption tests. A mixture of phages C<sub>341</sub> and  $\epsilon^{34}$  *vir*, each at  $1 \times 10^7$ /ml, was added and samples were taken after 2, 5, and 10 minutes. These were diluted 1:100 in broth with chloroform (to kill the bacterial cells) and plated 30-40 minutes later; thus, only the free phage was assayed. Phage  $\epsilon^{34}$  *vir* was assayed on strain A *do* and phage C<sub>341</sub> was assayed on

strain A, adding 2 drops of serum anti- $\epsilon^{15}$  (1:50) to each plate. Most of the phage  $\epsilon^{15}$  produced by bacteria lysing during the adsorption test is eliminated by the serum; even in controls with no serum added, however, the phage  $\epsilon^{15}$  present does not interfere with the assay of phage  $C_{341}$ . The adsorption rate constants for strain A and A( $\epsilon^{15}$ ) were measured in the same experiments.

The results of such an experiment are shown in Table 5. The adsorption rate constants are given as the means of the values derived from the platings at 2, 5, and 10 minutes. This procedure is justified for  $\epsilon^{34}$  *vir*, where the adsorption is generally a simple exponential function of time; less so for phage  $C_{341}$ , which is adsorbed more rapidly in the first few minutes. Table 5 also gives for comparison the proportion of phage-carrier cells found in the same experiments. It is clear that the adsorption capacity per cell for phage  $C_{341}$  decreases rapidly after infection, while the adsorption capacity for phage  $\epsilon^{34}$  *vir* appears and increases to a maximum. Later, the adsorption capacity for  $C_{341}$  reappears and increases again, while that for  $\epsilon^{34}$  *vir* rapidly decreases. As expected, the timing of these changes in adsorption rates parallels closely that of the changes in susceptibility to the phages, as can be seen by comparison with Table 2 and Fig. 1.

More interesting are the quantitative data. The adsorption rate for  $\epsilon^{34}$  *vir* reaches the maximum value of  $1.8 \times 10^{-9}$  at 150 minutes, that is, when the phage-carrier cells are already reduced to 24%. This maximum rate is the same as the rate for the established lysogenic strain A( $\epsilon^{15}$ ) tested under the same conditions, that is, grown in broth containing anti- $\epsilon^{15}$  serum (1:400) for 2 hours. The decrease in adsorption rate for  $\epsilon^{34}$  *vir* thereafter is such that at 300 minutes the rate is only about  $\frac{1}{10}$  the maximum rate per cell. In the interval between 150 and 300 minutes, the adsorption rates observed correspond closely (see Table 5) to the rates calculated by assuming that the population consists of two fractions: one, lysogenic, comprising 8% of the population, with maximum adsorption rate of  $1.8 \times 10^{-9}$  per cell; the other, with adsorption rate per cell corresponding to the maximum rate divided by the number of cells derived from each cell at 150 minutes. We interpret this as showing that in the cells without phage  $\epsilon^{15}$  the receptors for  $\epsilon^{34}$  *vir* that have been formed are diluted and distributed among the progeny cells but are not destroyed or otherwise inactivated. Note that in this period the proportion of cells susceptible to phage  $\epsilon^{34}$  *vir* decreases from 90 to 8%.

The situation is different with regard to phage  $C_{341}$ . Here the adsorp-

TABLE 5  
ADSORPTION RATE OF PHAGES  $C_{341}$  AND  $\epsilon^{34}$  *vir* ON PROGENY CELLS FROM BACTERIA INFECTED WITH  $\epsilon^{15}$

Time after infection	Cells/ml <sup>a</sup>	Phage carrier cells (%)	Adsorption rate constant per cell per minute for phage $C_{341} = k_c$		Adsorption rate constant per cell per minute for phage $\epsilon^{34}$ <i>vir</i> = $k_{34}$	
			Observed	Calculated <sup>b</sup>	Observed	Calculated <sup>b</sup>
30	$4.8 \times 10^7$	—	st.		—	
60	$1.0 \times 10^8$	90	$2.6 \times 10^{-9}$	$2.9 \times 10^{-9}$	$1.1 \times 10^{-9}$	
120	$5.2 \times 10^8$	62.5	$3.6 \times 10^{-10}$	$5.6 \times 10^{-10}$	$1.7 \times 10^{-9}$	
150	$8.9 \times 10^8$	24.5	$<1 \times 10^{-10}$	$3.3 \times 10^{-10}$	$1.8 \times 10^{-9}$	
180	$2.1 \times 10^9$	8.1	$3.6 \times 10^{-10}$		$7.7 \times 10^{-10}$	$8.6 \times 10^{-10}$
240	$1.2 \times 10^{10}$	6.8	$1.2 \times 10^{-9}$		$4.0 \times 10^{-10}$	$2.6 \times 10^{-10}$
300	$8.2 \times 10^{10}$	7.8	$1.9 \times 10^{-9}$		$1.9 \times 10^{-10}$	$1.6 \times 10^{-10}$

Strain A

$6 \times 10^{-9}$  in  
broth or  
in anti- $\epsilon^{15}$  serum

Strain A( $\epsilon^{15}$ )

$3 \times 10^{-9}$  in  
broth;  
 $1.8 \times 10^{-9}$   
in anti- $\epsilon^{15}$   
serum

<sup>a</sup> Values refer to the initial mixture of phage and bacteria.

<sup>b</sup> Calculated on the assumption of dilution of receptors by a factor of 2 at each cell generation (see text).

tion rate constant per cell decreases between 0 and 150 minutes from  $6 \times 10^{-9}$  to less than  $1 \times 10^{-10}$ . This decrease is more rapid than calculated from simple dilution of existing receptors (see Table 5). It is possible that the results may be affected by presence of the anti- $\epsilon^{15}$  serum. Although this serum does not interfere with adsorption of phage  $C_{341}$  by strain A, it cannot be excluded that it might affect the adsorption by bacteria with rapidly changing surface structure. There is additional evidence, however, that an actual loss of effective receptors for  $C_{341}$  takes place. The growing cells of strain A can adsorb at least 150 particles of phage  $C_{341}$  per cell, as shown by additional experiments. If simple dilution took place, after 3 generations each cell should have, on the average,



about 20 receptors and, after 5 generations, about 5 receptors. Yet, more and more cells become totally resistant to phage  $C_{341}$  in the killing tests, in which the phage is used in large excess. Here, too, it is difficult to exclude a possible role of antibody in anti- $\epsilon^{15}$  serum in preventing adsorption of and killing by phage  $C_{341}$ . This question will have to be solved experimentally before we can conclude with certainty that the receptors for phage  $C_{341}$  are actually destroyed or otherwise inactivated during the first 2 hours after infection.

*Coexistence and interactions of antigens 10 and 15 and of receptors for phages  $\epsilon^{34}$  and  $C_{341}$ .* The tube agglutination tests have indicated that early after infection most cells carry both antigens 10 and 15. The results of phage sensitivity and phage adsorption tests likewise show that the receptors for phages  $\epsilon^{34}$  *vir* and  $C_{341}$ , whose presence is closely associated with that of antigen 15 and antigen 10, respectively, are also present together in cells descended from  $\epsilon^{15}$ -infected bacteria. In fact, the data in Table 3 show that the sum of the proportions of cells killed by either  $\epsilon^{34}$  *vir* or  $C_{341}$  at 30, 60, and 90 minutes were 173, 191, and 173%, respectively. Thus, at least 73 to 91% of the cells must have receptors for both phages.

This does not mean that both antigens or both receptors can be synthesized in the same cell. All our results are compatible with the hypothesis that a cell can only form, either antigen 10 and receptor for  $C_{341}$  ("complex 10"), or antigen 15 and receptor for  $\epsilon^{34}$  *vir* ("complex 15"), as though the two synthesizing mechanisms were mutually exclusive. Formation of complex 15 is initiated rapidly by infection with  $\epsilon^{15}$ . Formation of complex 10 is initiated by the removal of phage  $\epsilon^{15}$  during segregation, but is apparently delayed for several generations, at least as tested by the reappearance of receptors for phage  $C_{341}$ . The possible interpretations of the competition and of the delay will be discussed later.

An unexpected observation was made when the  $\epsilon^{15}$ -infected cells were exposed at various times to a mixture of  $\epsilon^{34}$  *vir* and  $C_{341}$  phages at the same titers as used singly. The results, shown on Fig. 4, should be compared with those of Figs. 2 and 3. It was expected that all cells would at all times be killed by the mixture and that the rate of killing would be at least as fast as that by either phage alone. Instead, it is seen that at least at 60 minutes, the mixture of phages kills fewer cells and more slowly than phage  $C_{341}$  alone. This puzzling result may reflect an inhibition by  $\epsilon^{34}$  *vir* of the adsorption of phage  $C_{341}$  by 60-minute cells. Experi-

ments to test such an inhibition directly, however, gave erratic results and allowed no definite conclusion to be reached.

*Immunity to phage  $\epsilon^{15}$  vir.* Immunity of bacteria stably lysogenic for prophage  $\epsilon^{15}$  to lysis by superinfection with  $\epsilon^{15}$  *vir* cannot be tested because  $\epsilon^{15}$  and  $\epsilon^{15}$  *vir* are not adsorbed by cells lysogenic for  $\epsilon^{15}$ . A test can be made, however, during the period of segregation. The results of such tests are included in Table 2. It is seen that at 60 minutes all cells survive exposure to  $\epsilon^{15}$  *vir*, while at 180 minutes only about half survive. The failure to be killed by  $\epsilon^{15}$  *vir* may reflect either immunity or absence of receptors. Since at 60 minutes all cells are still sensitive to  $C_{341}$ , the resistance to  $\epsilon^{15}$  *vir* is probably a reflection of immunity. The results at 180 minutes show that receptors for  $C_{341}$  and to  $\epsilon^{15}$ , although they are both present in cells of strain A and absent from cells of strain A( $\epsilon^{15}$ ), are presumably not identical. This is in agreement with the observation that strain A( $\epsilon^{15}$ ) can adsorb  $\epsilon^{15}$  but not  $C_{341}$  (Uchida *et al.*, 1956; Uetake, 1957).

*Tests for antigens on protoplasts.* Zinder (personal communication) has detected antigen 1 in protoplasts of *S. typhi murium*. It seemed worth while testing whether antigen 10, which is not detectable in whole cells

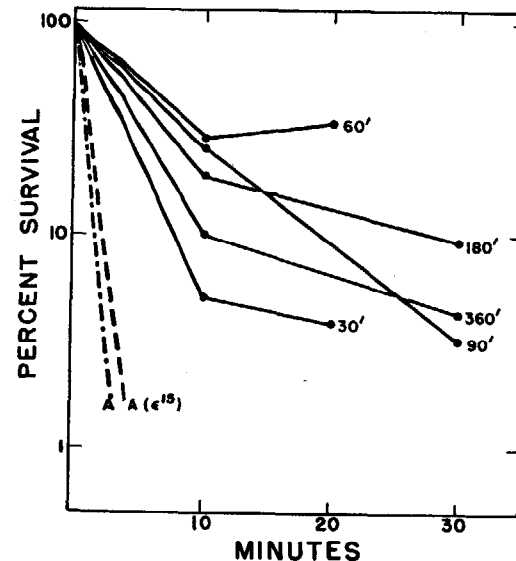


FIG. 4. The survival of bacteria exposed to a mixture of  $C_{341}$  and  $\epsilon^{34}$  *vir*. Notations as in Fig. 2.

of strain A( $\epsilon^{15}$ ), may still be present on the protoplasts, that is, on cells without cell walls.

Protoplasts were prepared from cells A and A( $\epsilon^{15}$ ) by the method of Fraser and Mahler (1957) and Fraser *et al.* (1957). Over 90% of the cells were transformed into protoplasts, that is, made round, nonviable, and sensitive to disruption by osmotic shock. They were washed and resuspended in a stabilizing medium (0.2% NaCl with 5% crystalline bovine serum albumin and  $10^{-2}$  M  $\text{MgSO}_4$ ) and tested for agglutinability by anti-10 and anti-15 sera. The mixtures were incubated at 37° for 2 hours and at 5° overnight. The results were clear-cut. Protoplasts from strain A cells are agglutinated by anti-10 serum only; those from strain A( $\epsilon^{15}$ ) are agglutinated by anti-15 serum only. The agglutination end point for the protoplasts is about the same as for intact cells in the same medium. The failure to reveal antigen 10 in the protoplasts of A( $\epsilon^{15}$ ) does not exclude, of course, that this antigen may be present in some masked form in the cells of this strain.

#### DISCUSSION

Infection of *S. anatum* strain A with phage  $\epsilon^{15}$  results in a rapid change in properties of the bacterial surface, recognizable as formation of somatic antigen 15, disappearance of antigen 10, acquisition of receptors for phage  $\epsilon^{34}$ , and loss of receptors for phage C<sub>341</sub>. Although the various properties are recognized by different tests and may reflect different details of specificity in the surface structures of the bacteria, their pattern of formation and disappearance following infection with phage  $\epsilon^{15}$  is consistent enough to justify for purposes of discussion the assumption of two alternative sets of characters, complex 10 and complex 15, each responsible for the antigenic and the phage receptor properties. Complex 15 is genetically determined by the activity of phage  $\epsilon^{15}$ ; complex 10 by its absence.

It should be realized that complex 10 and complex 15 are defined operationally only by the changes that the component properties undergo during lysogenization by phage  $\epsilon^{15}$ . Although the receptor for phage  $\epsilon^{15}$  is present in strain A and absent in strain A( $\epsilon^{15}$ ), we do not include it as part of the definition of complex 10, because its changes during lysogenization have not been investigated in detail. The fact that receptors for phage C<sub>341</sub> can be absent from cells with antigen 10 (Uchida *et al.*, 1956) does not invalidate the inclusion of these receptors in defining complex 10 with regard to the function of phage  $\epsilon^{15}$  as genetic determinant.

The most remarkable finding is the extremely rapid appearance of complex 15 in the infected bacteria and its relation to the activity of phage  $\epsilon^{15}$  in any one of its forms. By the agglutination test, complex 15 can be detected within 5 minutes after infection. After 30 minutes, when agglutination by anti-15 serum is almost as extensive as with cells of A( $\epsilon^{15}$ ), about 75% of the cells can already be killed by phage  $\epsilon^{34}$  *vir*, and by 60 minutes the average amount of  $\epsilon^{34}$  receptors, measured by the rate of phage adsorption per cell, is over half the maximum.

The rapidity of these events makes it possible to detect them by the agglutination test in cells destined to lysis, using as determining phage the mutant  $\epsilon^{15}$  *vir* instead of  $\epsilon^{15}$ . This has allowed us to prove that complex 15 is formed equally well in the lysis-awaiting cells. Hence, the determination of complex 15 does not require the function of a stable prophage, which may be part of the bacterial chromosome (see Bertani, 1957). The assumption of such a requirement has been present in the expression "lysogenic conversion," by which the occurrence of novel properties in lysogenized bacteria has been named. At least for the antigenic change produced by phage  $\epsilon^{15}$  in *Salmonella*, our results show that, provided suitable tests are available, the phage-determined properties can be found even in bacteria that carry the determining phage in the vegetative state. Additional evidence supporting this conclusion is being obtained with other phage-controlled antigenic conversions of *Salmonella* (Zinder, personal communication; Fraser, Luria and Burrous, unpublished).

It has been pointed out before (Luria, 1954) that all alterations in the patterns of biosynthesis in phage-infected bacteria may be considered as an expression of the genetic function of phage, even though the phenomena related to production of mature, infectious phage are not obviously recognizable as bacterial heredity (unless this is extended to include virus infection as infectious heredity). An important example is the recently discovered enzyme that synthesizes hydroxymethyl-deoxycytidylic acid in *E. coli* cells infected with phage T2 (Flaks and Cohen, 1957). This enzyme is present only in phage-infected cells. A difference between the functions of vegetative phage and of prophage, which might be considered as a form of "position effect," is observed in the failure of the prophage *as such* to initiate phage maturation in the host cell, as well as in differences in immunity and phage resistance patterns between lysogenic cells and cells with vegetative phage (S. Lederberg, 1957). It may be recalled that abortive transduction (Stocker *et al.*, 1953; Stocker, 1956; Lederberg, 1956; Ozeki, 1956) has already

indicated that cell functions can be controlled by newly introduced, unestablished fragments of genetic material.

The fact that complex 15 is formed in infected cells during the vegetative growth cycle of phage  $\epsilon^{15}$  removes one of the criteria used to distinguish between conversion and transduction. In conversion, a phage has been supposed to confer the new character to the lysogenized cells even when previously grown on cells that did not have the character in question. Since the character "complex 15" presumably appears in all cells where  $\epsilon^{15}$  grows, the distinction between conversion and obligatory transduction becomes a matter of definition. In fact, high frequency transduction by phage  $\lambda$  is apparently mediated by defective phage particles that have incorporated into their own genome a fragment of bacterial genome (Arber *et al.*, 1957). A converting phage may have a similar origin. Alternatively, a converting phage may be a fragment of bacterial genome that has acquired by mutation a viral function, that is, the ability to control its own maturation into an infectious particle.

The loss of receptors for phage  $\epsilon^{15}$  upon acquisition of complex 15, determined by that phage, exemplifies a situation in which a lysogenic bacterium fails to adsorb the phage related to its prophage. This situation is apparently common, although by no means general, in *Salmonella* (see Boyd, 1954; Uchida *et al.*, 1956). The frequent association between phage activity and genetic control of surface constituents in a fragment of genetic material may not be fortuitous. We may speculate as to its relation to the ability of the phage genome to control the synthesis of the specific apparatus by which the phage particles will combine with the surface receptors of the host cell.

The change from complex 10 to complex 15 is not simply a change from the expression of one genetic potentiality of the host cell to another potentiality, triggered by the infecting phage. The eventual loss of complex 15 in nonlysogenic segregants from infected cells is proof that the phage genome is required to maintain the new character. In preliminary tests, cells exposed to phage  $\epsilon^{15}$  inactivated by a heavy dose of ultraviolet light showed no antigenic changes. With phage that had received a lower dose of ultraviolet light some antigen 15 appeared. It should be possible to decide by quantitative studies whether the antigen-controlling function can be dissociated from the reproductive function of the phage.

The cells converted from complex 10 to complex 15 must retain the genetic factors for complex 10 since the strains lysogenic for  $\epsilon^{15}$  revert

specifically to the antigenic form 3,10 when they lose the prophage (Uetake *et al.*, 1955). These nonlysogenic derivatives can be isolated by cultivation in presence of anti-15 serum. Phage  $\epsilon^{15}$  appears to introduce an additional dominant genetic element rather than to replace it by a different one.

Bruner and Edwards (1948) reported the isolation of variants belonging to group E<sub>2</sub> (antigens 3,15) by cultivation of E<sub>1</sub> cells in media with anti-10 serum. Their anti-10 serum had been produced by absorption with heavy suspensions of bacteria belonging to group E<sub>2</sub>. These may have contained converting phage. When the experiment was repeated using anti-10 serum prepared by absorption with washed, killed cells of group E<sub>2</sub>, the antigenic change was not observed (Iseki and Sakai, 1953a; Uetake, unpublished experiments).

Our experiments have shown that complex 15, although determined by phage  $\epsilon^{15}$ , can be present in bacteria that by segregation have lost the phage genetic material, at least as detectable by production of phage or of specific immunity in their progeny. In the transition period the cells have both complex 10 and complex 15, as shown by agglutination and phage-sensitivity tests. Such a coexistence has already been reported, presumably as a permanent condition, in *S. lexington* and *S. macallen* infected with phage  $\epsilon^{15}$  (Uetake *et al.*, 1955; Nakagawa, 1955). In *S. anatum*, however, the coexistence is a transient one, observed both during the disappearance of complex 10 in the recently infected cells and during its reappearance in cells that have segregated away the phage  $\epsilon^{15}$ . What is the mechanism of these replacement phenomena?

The results on the receptors for the test phages, C<sub>341</sub> and  $\epsilon^{34}$  *vir*, are compatible with the assumption that, following infection with phage  $\epsilon^{15}$ , the existing complex 10 disappears faster than by simple dilutions among the progeny cells, whereas in cells that lose phage  $\epsilon^{15}$  the newly formed complex 15 is simply diluted away. The results are not conclusive, because the experiments may be complicated by unrecognized effects of the experimental conditions on adsorption of the test phages, as explained in the experimental section. If the complex 10 initially present actually disappears, it may either decay by intrinsic instability, or be sloughed off by the host cells, or masked by complex 15, or actually transformed into complex 15. These possibilities should be amenable to test by chemical isolation and isotope transfer experiments on the somatic antigens. Additional information should be obtained by analysis of other conversion phenomena observed in the E group of *Salmonella*

(Uetake *et al.*, 1955) as well as in the B group (Iseki and Kashiwagi, 1955; Terada *et al.*, 1956). The observed transitions between alternative cell properties in the phase of segregation of phage elements should provide a useful material for the study of gene action. It may, in fact, be ultimately possible to detect various gene products—the antigen itself and its component parts, as well as enzymatic or otherwise functional constituents of the antigen-forming system—and to relate their presence, amount, and function to the actual presence of the determining genetic element in a cell or in its direct ancestors.

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